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(54) Regulated genes by stimulation of chondrocytes with 1L-1beta

(57) The present invention refers to the novel use of osteopontin, calnexin and TSG-6 gene product in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1β and their use in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues.

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Description

The present invention refers to the novel use of osteopontin and calnexin in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1β and their use in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues.

Among the diverse biological effect of interleukin-1 (IL-1), are its actions on the metabolism of many connective tissue cell types including articular chondrocytes. IL-1 inhibits proteoglycan (PG) synthesis by chondrocytes and stimulates production of prostaglandin E_2 and metallo-proteinases capable of degrading matrix macromolecules. From experimental results, and from findings of IL-1, PG fragments and proteolytic enzymes in inflamed joints, it was deduced that IL-1 plays a role in cartilage degradation in osteoarthritis and rheumatoid arthritis (Benton HP & Tyler JA. 1988, Biochem, Biophys. Res. Comm. 154, 421-428; Aydelotte MB et al. Conn. Tiss. Res. 28, 143-159; Wood DD et at., Arthrithis Rheum. 26, 975-983; Lohmander LS et al., Trans Orthrop. Res. Soc. 17, 273). Matrix metalloproteinases are potential candidates for drug interaction at the enzyme level, but relevant molecular targets interfering with earlier processes leading to cartilage degradation are still lacking. Therefore, one objective of the present invention was to identify potential targets for drug modification of IL-1β induced cartilage degradation on the RNA level of human articular chondrocytes from osteoarthritic cartilage.

As an initial attempt to investigate differentially expressed genes in diseased cartilage, total RNA from IL-1β stimulated and unstimulated human chondrocytes was subjected to differential display of mRNA by reverse transcription and polymerase chain reaction (DDRT-PCR). This method can be used to identify and isolate those genes that are differentially expressed in two cell populations (Liang P & Pardee AB 1992, Science 257, 967-971; Liang P et al., AB 1993, Nucl. Acids Res. 21, 3269-3275; Bauer D et al. 1993, Nucl. Acids Res. 21, 4272-4280). The key element is to use a set of oligonucleotide primers, one hybridizing to the polyadenylated tail of mRNAs, the other being arbitrary decamers that anneal at different positions relative to the first primer. mRNA subpopulations defined by these primer pairs are amplified after reverse transcription and resolved on DNA sequencing gels. Band patterns are created, which are characteristic for each RNA population extracted from the cell population under study. For example, 100 different primer combinations should generate a total of approximately 10,000 PCR products for each population, which should represent about the half of all expressed cellular genes. A comparison of the band pattern obtained from two cell populations reveals differentially displayed bands which correspond to differentially expressed genes. Subsequently, differentially displayed bands can be extracted from the gel, reamplified, subcloned and sequenced.

Due to its extreme sensitivity, the appearance of artifactual bands is an inherent problem of the DDRT-PCR method used according to the present application. An additional problem is also the evaluation of complex gene expression patterns. Yet another problem of the present invention is that only minute amounts of RNA are available.

Therefore, it was particularly surprising that the DNA TAU1/1 with the sequences

TAU1/1(1)

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ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC ATCCCCGTTT      60
CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG GAAGGACAGT TATGAAACGA     120
GTCAGCTGGA TGACCAGAGT GCTGAAACCC ACAGCCACAA GCAGTCCAGA TTATATAAGC     180
GGAAA                                                                 185
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and

TAU1/1(2)

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CTAAATGCAA AGTGAGAAAT TGTATTTTTT CTCCTTTTAA TTGACCTCAG AAGATGCACT      60
ATCTAATTCA TGAGAAATAC GAAATTTCAG GTGTTTATCT TCTTCCTTAC TTTTGGGG       118
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and the DNA TTU2/2 with the sequence

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AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT TGTTTTCTTA    60
TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG CATGTTTTTC TTCATAGATA   120
CCCGTTTTGG GGTTTTTGCG TCGGAAGATG AATGGCATTT ATAGTCCTCT CCACATTTAT   180
CTG                                                                 183
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are 100 % identical to human osteopontin cDNA and 97.2 identical to human calnexin, respectively. This demonstrates that the experimental approach of the present invention worked efficiently, i.e. the use of 100 different primer combinations (25 oligodecamer primers, $4T_{12}$MN-primers) generated a total of approximately 10.000 PCR products for each population which represent 53 % of all expressed cellular genes. 123 PCR bands out of 10.000 appeared as differentially expressed bands. 53 of the original 123 PCR bands were reproducibly displayed by comparing the PCR band patterns from two patients; of those 68 % arose from IL-1β stimulated chondrocytes.

It was further found that osteopontin which is a secreted highly acidic phosphoprotein of 32 kd (Denhardt and Guo (1993) FASEB J. 7, 1475-1482) is surprisingly downregulated in IL-1β stimulated human chondrocytes. This means that osteopontin is involved in IL-1β related diseases of connective tissues, in particular osteoarthritis.

Osteoarthritis is characterized as a slowly progressing matrix degeneration with continuing degradation of collagens and proteoglycans and subsequent release of matrix fragments into the synovial fluid. Any disturbance of the normal chondrocyte matrix interactions, for example through a loss of osteopontin, could cause an altered signaling through the integrin $alpha_v beta_1$ and thus changed cellular responses leading to early steps of matrix degradation.

Therefore, one embodiment of the present invention is the use of osteopontin itself or parts thereof, antibodies against it or nucleic acids such as DNA or RNA or parts thereof coding for osteopontin or parts thereof in the diagnoses, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. According to the present application the term "parts" means either at least 8, preferably 12, in particular 15 amino acids in case of proteins or 6-100, preferably 10-40, in particular 12-25 nucleic acids in case of DNA or RNA as hybridization probes. The methods of diagnosing such diseases will be described infra. In addition, quantification on the protein level is possible with osteopontin specific antibodies on Western blots, in immunochemistry, FACS analysis or ELISA based assay systems. The present invention refers also to a diagnosis aid or a pharmaceutical for such use. Osteopontin can be produced for example recombinantly through expression in procayotes, in insect cells in mammalian cells or in mammalian cells using Vaccinia as detailed in Ausubel et al. 1994 [Current protocols in molecular biology, Chapter 16, John Wiley & Sons, Inc]. The cDNA of Osteopontin is e.g. disclosed in Young et al. (1990), Genomics 7, 491 - 502.

Antibodies against osteopontin can be generally produced for example by the method of Neil GA & Urnovitz HB (Trends in Biotechnology, 6, 209-213, 1988) or Köhler G & Milstein C (Nature, 256, 52-53, 1975).

Also calnexin which is an integral membrane protein of 88 kd (Bergeron et al. (1994) TIBS 19, 124-128) is surprisingly downregulated in IL-1β stimulated human chondrocytes compared to unstimulated chondrocytes. This means also that calnexin is involved in IL-1β related diseases of connective tissues, in particular osteoarthritis. In addition, a downregulation of the calnexin synthesis would cause a reduced amount of correctly and completely folded proteoglycans because calnexin is a new type of molecular chaperone that associates with incompletely folded proteins such as proteoglycans. Proteoglycans are highly glycosylated glycoproteins which are of central importance for the maintenance of the cartilage tissue integrity.

Hence, an additional embodiment of the present invention is the use of calnexin itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Calnexin can be produced for example recombinantly as described above for osteopontin. The cDNA of Calnexin is e.g. disclosed in Galvin et al. (1992), Proc. Natl. Acad. Sci. USA 89, 8452 - 8456. The production of said antibodies are also generally described above.

Potential role of identified cDNA fragments in IL-1 mediated cellular processes TSG-6

A homology search in the GenBank and EMBL databases revealed a 99.5 % sequence indentity of fragment TAU7/2(c) with the gene coding for human TSG-6. TSG-6 (TNF stimulated gene 6) was originally isolated by differential cDNA library screening as a TNF induced gene sequence from human fibroblasts (Lee et al., 1990). It was further characterized by Lee et al (1992) as a TNF and IL-1 inducible, secretory, 39 kDa glycoprotein with extensive sequence homology with a region implicated in hyaluronate binding, present in cartilage link protein, proteoglycan core proteins,

and the adhesion receptor CD44. With the ability to bind HA and with the most extensive sequence homology to CD44, TSG-6 belongs to the hyaladherin family. Wisniewski et al. (1993) detected high levels of TSG-6 protein in synovial fluids of patients with various forms of arthritis. Six normal control patients did not contain detectable TSG-6 protein in their joint fluid, whereas joint fluids from nine rheumatoid arthritis patients contained high, moderate or low levels of TSG-6. Two patients with osteoarthritis had high levels of TSG-6 in their joint fluids. The apparent local source of TSG-6 in the joints are synoviocytes and chondrocytes (Wisniewski et al., 1993). Lee et al. (1992) speculated that TSG-6 could act as a competitive inhibitor of the interaction between CD44 and its ligand(s) and thus might influence the structural organization of the extracellular matrix of connective tissue, resulting in a destabilization of the proteoglycan aggregates.

Hence, an additional embodiment of the present invention is the use of TSG-6 gene product itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Fibronectin

A homology search in the GenBank and EMBL databases revealed a 100 % sequence identity of fragment TTO20/1(c) with the gene coding for human fibronectin.
Fibronectin is a 450 kd glycoprotein with various functions. It acts as an adhesive ligand, as growth or differentiation factor and has chemotactic properties. It is found in the extracellular matrix of most types of cells (Hynes R 1993. Fibronectins, In: Guidebook to the extracellular matrix and adhesion proteins. Editors: Kreis T, Vale R. Oxford University Press. 56-58). An enhanced accumulation of fibronectin and fragments derived from it are found in the synovial fluid and on the inflamed synovial and pannus surfaces in the knee joint of patients with rheumatoid arthritis (Dutu A, Vlaicu-Rus V, Bolosiu HD, Parasca I, Cristea A. 1986. Fibronectin in plasma and synovial fluid of patients with rheumatic diseases. Med. Interne 24, 61-68). Patients with osteoarthritis, as well, have greatly increased levels of fibronectin in their synovial fluid and on cartilage surfaces (Xie D-L, Meyers R, Homandberg GA. 1992. Fibronectin fragments in osteoarthritic synovial fluid. J. Rheumatology 19, 1448-1452). The intraarticular injection of fibronectin fragments causes a severe depletion of cartilage proteoglycans in vivo (Homandberg GA, Meyers R, Williams JM. 1993. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo. J. of Rheumatology 20, 1378-1382), which is explained by the induced relase of several proteinases, including stromelysin (Xie D-L, Hui F, Meyers R, Homandberg GA. 1994. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: Stromelysin plays a major role in chondrolysis. Arch. Biochem. and Biophysics 311, 205-212). At high concentrations, fibronectin fragments enhance cartilage catabolism through release of cytokines, including IL-1 (Homandberg et al., personal communication).

In respect to these published data, the upregulation of fibronectin by IL-1 can be regarded as a positive feedback regulation, enhancing the self destructive potential of chondrocytes and synoviocytes. With this, fibronectin expression is a direct pharmacological target.

In addition, the sequencing of differentially displayed PCR products discovered also unknown DNA fragments which correspond to differentially expressed genes with or without stimulation of chondrocytes with IL-1β.

Therefore, another embodiment of the present invention is a DNA containing a DNA selected from the group consisting of

TA08/2(2)

1	CCAAGTTTTT	CCAGCAACCC	CAAGGGAATA	CAGGGAGATC	AATGCACCA
51	AAATGGGAAA	AGAAAAATAC	TTCGATGCAA	TGAAACAAAG	CCTTTTTCCG
101	TTCAGTTTCC	ATAATTCAGT	GGTCAGTTTT	AAGGCTGCCA	CTTGGG

TA016/1(2)

1	GACACGAACA	CCACATATTT	TTATTGGAGG	CCCCATGGCT	CCTTGGAAGC
51	CATTTTGGAA	CCAAGGGGAC	CCACCTTTTT		

TA016/2(2)

1	CTAAATATAT	TCTCTAACAA	GTTAATCTCT	TTCAAATCTA	TAGATAAAAC
51	TAAAAGGATA	AGGAACCAAG	GTTTAACCGA	CCTAGCCAAT	TATGGCAATC
101	ATACTTGCTT	TTTAG			

TA017(C)

1	CATGAAATAT	TTCTTGAGGT	AATAAGCTTT	TACCAAGCTT	ATATTTTTGG
51	GCAATTCAGT	TACAATGAGA	AAAAAACACA	CCAAAAGACC	AAAAATTTTA
101	AAAACTCACT	TTTCTTGCAA	TCATAGACAT	TTGCATTATT	ATAGAACATT
151	CAAACAAGTT	AGGTGGATAA	TTATTGTCTA	TAGATAAATA	CGATGCAATT
201	TTTTTAATGT	ATGACCGATA	CTCCGTATAT	ACTTAGATAA	CTTATCCAGA
301	AACCTCAACT	GTATTGAACA	TTGCTGAGAG	AAATCAACAA	TAATTTTAAC
351	AGATATGATG	ACAGNAAAAA	TTGATTGCAT	ATCTTTTTGC	ACTAAAACTT
401	TTATATTTAT	TT			

TA019(C)

1	AGAGCAGGGG	TATTTCNCGG	TTCATACCGC	CATGGCTTAA	GAAGCAAAAG
51	TCATATACCT	TAGTAGTGGC	AAAGATNGAG	GAGATAAAAA	AGAGCCTACC
101	CAAGCTGTTG	TTGAAGAACA	GGTCTTAGAT	AAAGAGGAAC	CCTTCCAGAA
151	GNACAGAGAC	AGGCTAAGGG	TGATGCTGAG	GAAATGGCTC	AGAAGAAACA
201	AGAGATTAA				

TAU 1/1(2)

1	CTAAATGCAA	AGTGAGAAAT	TGTATTTTTT	CTCCTTTTAA	TTGACCTCAG
51	AAGATGCACT	ATCTAATTCA	TGAGAAATAC	GAAATTTCAG	GTGTTTATCT
101	TCTTCCTTAC	TTTTGGGG			

TAU 1/1(1)

1	ACATCACCTC	ACACATGGAA	AGCGAGGAGT	TGAATGGTGC	ATACAAGGCC
51	ATCCCCGTTT	CCCAGGACCT	GAACCCGCCT	TCTGATTGGG	ACAGCCGTGG
101	GAAGGACAGT	TATGAAACGA	GTCAGCTGGA	TGACCAGAGT	GCTGAAACCC
151	ACAGCCACAA	GCAGTCCAGA	TTATATAAGC	GGAAA	

TAU1/2(2)

1	CCGGAATGGG	GAGCAAACTA	TAAGAACCGG	GACCAGTTTC	CTCTCTTTGT
51	GCCCTAGTTC	CCCCTCCTTT	GTATACACCC	TCCATCCTGA	ATAGACTCTG
101	GTTCTCAGCG	TAACACCGAC	AACATTCAAT	CCTGTAGAGA	AACAAATGTT
151	AGCTCAGAAG	GACACAGCCT	TTGAATCATC	AGAGAGTT	

TAU 7/1(2)

1	GTTAAGAATA	ACTAAATAAA	AGTTTTAATT	AATTTAGGAA	TATAAAAAAC
51	TATTAACATT	TAATTTTATA	ACTGTATCTG	CCAAGCAACT	TTAAATATAA
101	TTTATTTACC				

TAU 7/1(1)

1	CACGCAATGT	GAAATAGGCA	CATAGGAAGA	ATGGGGAAAC	CATCCCCTCA
51	AGCATTTATC	CTTTGAGTTA	CAAGCAATCC	AATTACACTC	TTTTAGTTAT
101	TTTTAAATGT	ACAGTTAGGT	TATTA		

TAU 7/2(C)

1	CCTTGAAGAT	GACCCAGGTT	NCTTGGCTGA	TTATGTTGAA	ATATAGACA
51	GTTACGATGA	TGTCCATGGC	TTTGTGGGAA	GATACTGTGG	AGATGAGCTT
101	CCAGATGACA	TCATCAGTAC	AGGAAATGTC	ATGACCTTGA	AGTTTCTAAG
151	TGATGCTTCA	GTGACAGCTG	GAGGTTTCCA	AATCAAATAT	GTTGCAATGG
201	AT				

TAU10(1)

1	GGAGATGACA	TTTGCTTTGG	GCAGAGGCAG	CTAGCCAGGA	CACATTTCCA
51	CTATAATTTT	ACAAAGTTAA	ATTTATAAGC	TAGCATTAAG	TAAAGTGAAG
101	TTCCAGCTCC	CTTGCTAAAA	ATAACTAGAG	GTAATAATTG	GTATTCAGGT
151	AACTCATTTA	CATCATAATG	TGTTGTGAAA	A	

TAU12/1(2)

1	TATAAAATAT	AAATTATATT	ATAAATCATG	TATTATTTAT	AAAATTATAT
51	TATAAATTTA	TAAAAATATA	AATTATATTT	TAGGCTTAAT	GTATAAGGAA
101	TATAAATTAT	TAATAAGCAT	ATGA		

TAU 12/1(1)

1	TGTAATTAAC	TGTNCTTGTA	GGTCTGTCTT	TTATACATGT	GTGAGTTTTT
51	CTTTACAATA	GATTCCTAGC	ATTGGGATTG	CTAGGTCAGA	TGGTATGCAC
101	ATTTGACATT	TTGATTGATA	GCACCAGATT	GCTTTGTTAA	AAAATTTTNN
151	TTTATAGTTT	ACATTATCTT	TGTACAATAG	ATGTTCTCTT	TCGAC

TAU 12/2(1)

1	GGGAAGTGAA	TTGAAAATAC	TTCTTTNTCA	ACATAATTTT	NGGGTTTTGA
51	AATTGTGTTT	GGGTTTTCAG	GAAATTGGTG	GTAATCTTGT	ATTAGACTGAA
101	AAAAAGTGAA	TTTTAAAATT	CTCAGTGAAG	AAGCAAATGA	TTTATTTTTC
151	ATAGA				

TAU12/3(2)

1	TGTTCTGGTA	ACTGTTCTAA	TTGTGTCTTT	GTTACTTCCA	GTGCAACCCT
51	TTCAGGTAAG				

TAU12/3(1)

1	CTAAAGAACT	TGGTATCTCT	ATTAAAGCAC	ACGAACCTCC	AAGGAAAATA
51	GAGCGATTTA	CTCTTCTCAT	ATCAGTGCAT	ATTTATAAGA	AGCACGGAGT
101	CA				

TAU13/1(1)

1	AGTCATCAAT	TCCTTTTTAT	CTGTAATTAC	ACATTTGTTT	TTATTTCAAA
51	GTAATTATAA	GGTGTTATAT	TGCATATAAT	CAGAAAACTA	AATGGAAATA
101	AAATTTTAGT	AAGCCCGGCC	CCTTTGACCG	ATACAGAAAA	CTTGA

TAU 13/3(2)

1	TATATGGCAG	TCTAAAGCAT	CAAAGATTTG	CATCAACATC	TTTCATTTTA
51	GACATCTCCT	TGCAATGTAA	AATATCATGT	ATCAACAACA	TCTGGTGCAA
101	ATCCATGAGT	CTAACTCGAC	ATTCATCTTA	GCTCGATTAT	TATTCCTTCG
151	TACAGTCGAT	GTAAACAATA	CAGAAAGAGG	ATTATTAAGA	ACAGTTT

TAU 13/3(1)

1	ATTCATGAAA	TGGTCTATAT	GCATGATATT	GTAAATTCGG	ACTCGAAACC
51	GAAACCAAGG	ATTCCGTTAC	AAAAATTCCT	TAATGCTGAG	AATGTTCTCA
101	CGCAAACAAC	ATCATGGACA	TTAAATTCAA	GATATGTGAA	TGTTAATTCT
151	GTCAATAAAG	TCAACGTAAA	GAGTAAAGTT	AAAAACAGTT	ATATCTNNNC
201	TGTCAATGAT	GAGTTTAGTT	TAACAGATGA	TGAATCAATT	CT

TCO 16/1(C)

1	CAAAGTGTTT	TTGGTTTTGA	GAGAGAGAGA	GATTGAGAGA	CAGAGAGAGA
51	GAGAGAAACC	AAGGGATCAT	GATAGTTATA	GTCAAATACG	AGGTTGGATT
101	ATCTTTTGAA	AATGTGTTGG	TTCTGTGATA	CAAGAGGAAG	CTAAGACATA
151	TCGTGGAAAC	ATCTCCCCCC	TCCACCTTAA	TATCAAGAAC	AAATTGTGGA
201	ATCTAATGTT	AATGAGAAGT	AGTTCCCCAC	TGTGTCAGAT	G

TCO16/2(C)

1	NCATCTGACA	CAGTGGGGAA	CTACTTCTCA	TTAACATTAG	ATTCCACAAT
51	TTNNNCTTGA	TATTAAGGNN	NNNNNGGGAG	ATCGTTTCAC	GATATCGTCT
101	TAGCTTCCTC	TTGTATCACA	GAACCAACAC	ATTTCAAAAG	ATAATCCTTC
151	CTCNNTTTGA	CTATAACTAT	CATGATCCCT	TGGTTCTCTC	TCTCTCTCTG
201	CTCTCTCATC	TCTCTCTCTC	TNAAAACNAA		

TCO17(C)

1	ACAGTAGTTA	GGAGTTTCTT	TACTTACAAA	ATCACTGGAA	ATGATTAAAT
51	TGCTTTTCCC	CCTCCCCAGA	GGTGCATTTT	TCTTATTTCC	ATATAGTAAA
101	GTTGAGCTTT	TACAGTGCAT	AATGTGACAT	TTGGAATGCT	TATCAACTGC
151	ATGTAAACAT	TAATAACCT			

TCO18(C)

1	GTAAATGGTA	TTANNNGCTG	AAGAAAAAAA	ATTTTTCAAG	ACCTCTGTTT
51	TTTAACTGAA	CTTTATCATT	GGCATTGTGG	GCTTTGAAGT	TGCTGGGATA
101	AATTAATATA	ATTAAATAAA	AGACTGAATT	TAATTGCAAA	AAAAAAAAAA
151	AACAATAAGT	GTGGTGAT			

TCU2/1(1)

1	AAGAAATTAT	CCAGTTATTT	ACAAGGCCAC	TGATATTTTA	AACGTCCAAA
51	AGTTTGTTTA	AATGGGCTGT	TACCGCTGAG	AATGATGAGG	ATGAGAATGA
101	TGGTTGAAGG	TTACATTTTA	GGAAATGAAG	AAACTTAGAA	AATTAATATA
151	AAGACAGTGA	TAAATACAAA	GAAGATTT		

TCU2/2(1)

1	CGGGTTAATA	TTATCCTCTA	GTATAAGTGA	ATTACTAGTT	TCTCTTTATT
51	TAGACAAACA	CACACACACC	AGATAATATA	AACTTAATAA	ATTATCTGTT
101	AATGTAGATT	TTATTTAAAA	AACTATATTT	GAACATTGGT	CTTTCTTGGA
151	C				

TCU9/1(2)

1	ACATAACAGC	TTTTATACAA	TGATAAGGAC	ATATCATTTG	TTTACAAAGA
51	AAGTCTAAAA	TTTCAAGAAC	ATTCAAAGAG	CTAACACAGT	AAAGGTCATG
101	CAAGTTCTAG	AATAGTGAAT	CATGACAGAA	CTCATTCATT	TTATCCTTTA
151	TCTCC				

TCU9/2(2)

1	AAGTATGGGT	AGCTAAATTT	GCATTAAATT	AAAAGTACAT	ATAATGCAAC
51	ACCACTCTAC	ATCTGTATAC	CTACGAATGT	ATGTGTACTA	CACACCCTTA
101	AAATGTTTTT	CAAAGTCTTA	ATATATTAGA	ACATGTTTTC	ATTTTTTCAT
151	GGGATGTTAA	TACTATTCTA	TGATTAAGAA	AATACTAG	

TCU10(2)

1	AATACAGTTA	TTCTAGCTTT	TCATATTCAA	TTTGAATGAT	CAGAAAAGTA
51	TATTAGTCAC	ACAGAATTAA	ATATTTTAGA	TAGTAAGAAT	C

TCU14(2)

1	GAAGTGAAAG	TCAGCCCTTT	AGCTATTATT	TATTGCTTTA	TTAGAGCAGA
51	GGGAAGTGAC	ACTCATTGCC	TTCACAGAGC	TCTGCAGAAA	TATATGCACA
101	GAGTGGTCAA	TGCCAACATC	TGAGTAAGTC	TTCCAAA	

TGO20(2)

1	CAGAACATTA	GGATTTATTC	CTTGATTAGT	TCAAATGATT	TCAACAGCTG
51	AATTCCTTGA	GATGTGTAAG	GCAGGTTGGT	CCTTTGGATG	GACTGTAGAC
101	TGAAACTTCC	TATAACTGTA	GTGATATGTA	CACAGCTACA	TAGCAAAGTG
151	CTTCATTATG	AAAATGAAGA	A		

TGO20(1)

1	CAGTGTGAGA	GTCTCATTTC	TATGCACAGT	GTTTCTCAGG	AGGATGGAGC
51	TAGTTAGCTG	TCTGTTGTCT	GTAGCCCAGC	TTGATAATGG	AACTATACAG
101	CGAAGAGACA	ATCTCTGGCA	AGTTTTTGTA	GAA	

TGU5(C)

1	TTAGAGTAAA	ATTCCAAATA	AATGCTTTGC	TCCAAAATTA	CACTAACCAG
51	GCTGGGTCTC	TATCATACAT	CTTCAATACC	CTCAAACCTA	GATTGTAAAG
101	TGAAAAAAGT	GATTAGCNNT	TCCATTTGTT	CATTCTGTCA	CTCACATTCT
151	TAGGCATTTT	AAGGATGAGC	AACCTTTGTT	TCAGAAAGGG	TAAGTAATTA
201	GCCCCCTGGA	GGTTACATAG	TTATAATTTA	GTCTTCAGAA	TCCGTTCGAA

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      251   GGGNNNNGTT  ACTATTTTTA  AGATAATTAG  AACCCACCTT  GTAGCAATAA
      301   AAGTTTTCTT  GTCTTTG

TGU8(2)
        1   GCGAAAGACT  AATCGAACCA  TCTAGTAGCT  GGTTCCCTCC  GAAGTTTCCC
       51   TCAGGATA

TGU9/1(2)
        1   TTAATGTTTA  AATACTACTT  TTTTTTCAAG  CTTGCCCTAG  ATACCAACTG
       51   TTTATCTAAC  ACACAATTCC  AGTGTTGCCA  AGCCTCATGC  CAATTTGAAG
      101   GGAACAGCCA  AAACTTATGC  ATTCATATAA  AAAGAGTCTC  TAGGCTCTTA
      151   TATCTACATT  ATAATTTTT

TGU9/2(2)
        1   GGAATAACAT  TTTTTTATGA  GGGAACCCTT  TAAAATGGAT  GCACACAGTG
       51   GCATTTTCTC  CTAGGCTCAA  AGCTGAGTAC  ACTCCCGTAA  TTTTAATAAT
      101   ATTTTAGGCA  AGTCCTATGA  CAATTATACC  AACAAGTTTC  TTCAACCCCA
      151   CCACCACCCC  ACCATCTCTA  TGC

TGU12(C)
        1   GGAGGAAGCT  TTATTTGGGA  AGAGTGCGGT  TCNNTCGGCC  CTGATCAGCT
       51   CTAGCCTGCC  CACCCCATCT  CAGCCAGGCG  GCTTTACTTC  TTCCTGAGCT
      101   TCAGGTCTTT  CTTCTTCCTG  ATTTCCTTGG  CCAGCTCCCC  AATCAATCTC
      151   CAGTACTCAT  TGAACTTGAG  CTCCGAGNCC  TGATTCACAT  CCAAGCTCTT
      201   CATCTTCT

TGU13/1(C)
        1   GGATGTGGTA  GTTGATCTTT  AATGCCCATT  CTAGGTCGGA  AAAATCCATG
       51   ATCCTAACTT  TTAAGAGAAG  GTTGGTAACT  CTACTTAGGA  CTTTTTTTTG
      101   TAAGAGGAAT  AATGTAGCCT  CACCCTTATC  TTTCTGGAAA  TGTTTAAACC
      151   ACTGAAATAT  GGAGATCAAA  TCCAGCTTAC  ACACTGGTAA  CTCAAATACT
      201   ATTTTTTTTT  TAAACTATCT  TTTCTAAACT  AATCACCCCT  CTTGTACATA
      251   GAACTTTCTA  TCTCAGTGCC  AATTCTTAGA  GGTTGATGCA  AACAGCTCTC
      301   CAGAGAGCCT  GTGCTATTGT  TC

TGU13/2(2)
        1   GGGGTGTACA  TTTTATTGGA  AACCTTAAAT  ACTGTTCAGA  AAGAATATAT
       51   CTTCAATCAA  GGTCTTGCCG  AGCCTACACA  GAAAAATGAA  GCTTTTTGGG
      101   TTAGGGGCAA  GGATATATAC  AGTACAGAGG  ACAAAGA

TTO16/2(C)
        1   ACATTCATTA  AAGATGAACT  TTCAGCATCT  TCACTTGAAG  ATCCATCAGA
       51   TGATTCTGAG  AGGCAGGTCT  CCCCCAAAAA  TCCACCGCAT  GTATTCTTTC
      101   GTTTAGAATC  TGAAAGCCTC  TTTCTTTTCA  GGCTTGATGA  CTCTTCTAAG
      151   GTATTTGTTA  TGCCTCTCTT  CTGGGTTTTT  CGTTTTGCCT  TATCAAGTAG
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```
      201   CTNAAATTCA  AACACCATGG  CAANAGAAAC  TGCTTCTAT

TTO20/1(C)
        1   CCACCAGCCT  ACTGATCAGC  TGGGATGCTC  CTGCTGTCAC  AGTGAGATAT
       51   TACAGGATCA  CTTACGGAGA  AACAGGAGGA  AATAGCCCTG  TCCAGGAGTT
      101   CACTGTGCCT  GGGAGCAAGT  CTACAGCTAC  CATCAGCGGC  CTTAAACCTG
      151   GAGTTGATTA  TACCATCACT  GTGTATGCTG  TCACTGGCCG  TGGAGACAGC

      201   CCCGCAAGCA  GCAAGCCAAT  TTCCATTAAT  TACCGAACAG  AAATTGACAA
      251   ACCATCCCAG  ATGCAAGTGA  CCGATGTTCA  AGACAACTGT  TTTAATAAAA
      301   GATTTACATT  CCAC

TTO20/2(2)
        1   TTGGTACCAC  AGTCACAGAA  CTGGGGGTCA  TTTTCTAGAT  GAAACAAACG
       51   GAACAAGTTC  TCTTCCAACA  AAGAAATGTA  CTGTAGAAAT  TAATTTCCTC
      101   CATGAATTTT  ATATATTGTG  TACAAATATA  AGGTATGTAT  CTGAATACAA
      151   AG

TTU2/1(2)
        1   CTAGAACTTC  CAAAGGCTGC  TTGTCATAGA  AGCCATTGCA  TCTATAAAGC
       51   AACGGCTCCT  GTTAAATGGT  ATCTCCTTTC  TGAGGCTCCT  ACTAAAAGTC
      101   ATTTGTTACC  TAAACCTTAT  GTGCCTTAAC  AGGCCAATGC  TTCTCG

TTU 2/2(C)
        1   AACCAGTATT  TCAAAACTAT  TATCTGGATT  CAAGATTAGT  GTGTAAAGAT
       51   TGTTTTCTTA  TCAGTAAAAT  AGGTCTTCAG  ATCTGCATCT  GGCCTCTTAG
      101   CATGTTTTTC  TTCATAGATA  CCCGTTTTGG  GGTTTTTGCG  TCGGAAGATG
      151   AAGTGCAGTT  TATAGTCCTC  TCCACATTTA  TCTG

TTU3(1)
        1   GGGTAGAAAG  CTGAATAATT  TATGAAGGAG  AGGGGTCAGG  GTTGATTCGG
       51   GAGGACCTAT  TGGTGCGGGG  GCTTTGTATG  ATTATGGGCG  TTGATTAGTA
      101   GTAGTTACTG  GTTGAACATT  GTTTGTTGGT  GTATATATTG  TAATTGAGAT
      151   TGCTCGGGGG  AATAGGTTAT  GTGATTAGGA  GTAGGGTTAG  GATGAGTGGG
      201   AAG

TTU 5/1(2)
        1   GACAAAAAAA  AAAAAACAGG  TTTTAAAGCT  AGAAATGAAA  AGCTACTTAA
       51   GTATCTTAAA  GGATAAGTTA  CTTTATTATA  CACTAGAAAC  ATACACAATA
      101   GCTGAAAACT  TAAAAAATCT  CACACTGCTG  AATGTCTCTG  CTGGCTG

TTU5/2(2)
        1   GCATCCATTG  TACATTGTTT  GGTTTGAGGT  TACCATGAGG  CCTGTAAATA
       51   CTATCTTATA  ATTTATTATT  TCAACCTGAT  AAAACTTAAC  ACTATTTGCA
      101   TAAACAAACA  AACGAAAA
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TTU9/1(1)
       1   TAAAATACTG  GTTCTTTTAT  TCTGCAATAT  TTTTAAAAAT  CACATTTTCA
      51   GCCAGGCGCA  GTTTCCCACA  CCTGTAATCC  GGCACTTTGG  GAGGCTGAGA
     101   TGGGTGGATC  ACAAGGTAGG  AGATCAAACA  TCCTGGCCAA  CATGGTGAAC
     151   CTGTTTACT

TTU9/2(2)
       1  CAAGTATGGG TAGCTAAATT TGCATTAAAT TAAAAGTACA TATAATGCAA
      51  CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCTT
     101  AAATGTTTCA AAGCTTAATA TATTAGAACA TGTTTTCATT TTCAGGGAG

TTU13(2)
       1  GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG
      51  TAAAATGAAA GAAATCATTT TTAACTCCTA AGATGT

TTU13(1)
       1  TGAATTAAAT GGACTCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT
      51  AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT
     101  CTTCTTTT
```

or an analog thereof. In accordance with the invention, the term "analog" includes nucleic acids which code for the same protein sequence due to the degeneration of the genetic code, for a protein having conservative amino acids substitutions or deletions that do not eliminate the characteristical feature of this protein, or for a protein having at least about 85 %, and more advantageously at least about 90 %, in particular 95 % amino acid sequence homology.

Other embodiments of the invention provide a vector containing said DNA and a host cell containing said vector.

According to the general knowledge one skilled in the art can also use said nucleic acids of the present invention as a hybridization probe to detect the corresponding genes in an organism or in a sample from on organism or gene mutations thereof.

Therefore, an additional embodiment is a method for isolating a gene which can be induced or repressed by treating chondrocytes that contain this gene by IL-1β containing the steps:

(a) hybridizing a DNA of the present invention under stringent preferably high stringent conditions against DNA or RNA containing said gene, preferably DNA or RNA isolated originally from chondrocytes, in particular human chondrocytes; and

(b) isolating this gene by methods known to a skilled person in the art.

According to the present invention the term "stringent conditions" means hybridization conditions comprising a salt concentration of 4 x SSC (NaCl-citrate buffer) at 62-66°C, and "high stringent conditions" means hybridization conditions comprising a salt concentration of 0,1 x SSC at 68°C. The length of the probes are 6-100, preferably 10-40, in particular 12-25 nucleic acids long.

Yet another embodiment is a process for expressing a gene isolated according to the above-described process containing the steps:

(a) cloning said gene into a suitable expression vector such as the pET series (Studier et al., 1990. Methods in Enzymology 185, 60) for procaryotic expression or the vector CDM8 for mammalian expression (Aruffo and Seed, 1987. Proc. Natl. Acad. Sci. USA 84, 8573) or any other expression system known to one skilled in the art; and

(b) expressing said gene in a suitable host cell such as BL21 series (Studier et al., 1990, supra) for procaryotic expression or COS, cells for mammalian expression (Aruffo and Seed, 1987, supra) or any other expression system known to one skilled in the art;

or a method for producing a protein containing the steps:

(a) culturing a suitable host cell, in particular the above mentioned, containing a vector, in particular an expression vector such as the vectors mentioned above which contains a DNA or a gene of the present invention; and

(b) isolating the expressed protein for example by ultrafiltration, precipitation with chaotropic agents such as urea or column chromatography on e.g. ion exchange chromatography columns as detailed in Ausubel et al. 1994 (supra).

A further embodiment is a diagnostic aid containing a DNA or parts thereof or a gene or parts thereof of the present invention. In particular, quantification of the genes can be achieved on the RNA level by Northern blotting with gene specific probes of the present invention or with gene specific primers in a PCR reaction. Such primers can be synthetically produced using the DNA sequences of the present invention or the sequences of the corresponding genes. Therefore, said nucleic acids are useful for the diagnosis of IL-1β related diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

These nucleic acids can also be used to evaluate the expression of certain genes in small cartilage biopsies and to use these ultimately as disease-specific markers and/or as predictive markers for disease progression of e.g. osteoarthritis. The hybridization conditions can be the same as described above.

Said nucleic acids, however, can also be used for the therapy against the diseases mentioned or for the production of a pharmaceutical.

Therefore, another embodiment of the present invention is also the use of said nucleic acids for the production of a pharmaceutical. For example, as described by Uhlmann & Peyman (Chem. Rev. (1990), 90, 543), Milligan et al. (J. Med. Chem. (1993), 36, 1923) or Stein & Cheng (Science (1993), 261, 1004) such nucleic acids can be used as antisense oligonucleotides or triple helix forming oligonucleotides for the inhibition of gene expression. This is in particular useful if such a disease is caused by the overproduction of a gene product which is directly or indirectly regulated by IL-1β in chondrocytes. The nucleic acids can additionally be modified in order to increase e.g. the stability against nucleases as described e.g. in the literatures mentioned above.

Finally, also the gene product itself produced by a method of the present invention can be used as a pharmaceutical.

In the following the invention is in particular described by the examples and tables:

Description of the Tables

Table 1 gives an overview on used primers and the complexity of the detected differences in expression.

Table 2 summarizes the result of the sequencing of differentially displayed PCR products after their elution from the sequencing gel, reamplification and subcloning into the pCRII vector. The sequences of TAU1/1(1) and TAU1/1(2) are 100 % identical to human osteopontin cDNA, the sequence of TTU2/2 is 97.2 % identical to human calnexin. bp = base pairs, IL-1 = Interleukin-1 stimulation, Stat. sig. score = statistical significance score: a feature of the BLAST database searching program. This score is determined using an implementation of Karlin's significance formula (Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natl. Acad. Sci. USA, 87:2264-2268), which calculates the Poisson probability that the observed sequence similarity will occur by chance based on the size and composition of the sequence database as well as on the size and quality of the match. The smaller this number, the more it is likely to see sequence similarities.

Examples

Cell culture

Articular cartilage specimen were obtained from two patients (male 65 years old and female 73 years old) undergoing total joint replacement surgery for osteoarthritis. None of these individuals had received treatment by radiation or chemotherapy. Articular cartilage slices were aseptically dissected from both femoral condyles, tibia plateaus and pattellae and subjected to sequential enzymatic digestion with pronase and collagenase as described (Häuselmann HJ et al. 1992, Matrix 12, 116-129) Since it is known that the alginate gel suspension system retains the chondrogenic phenotype [Lohmander LS et al. 1992, Trans. Orthop. Res. Soc. 17, 273.] 4 x 10^6 chondrocytes were suspended in low viscosity alginate (4 x 10^6 cells / ml 1,25 % w/v alginate in an isotonic buffered solution) and expressed through a 22gauche needle into 102 mM CaCl solution to form cell entrapping beads which are 1,5-3 mm in diameter and spherical. Alginate beads containing a total number of 2 x 10^7 cells were fed daily for the first three days with medium F12 / DMEM (50/50)

and 10 % fetal calf serum (Sigma) with 25 µg / ml ascorbate and 50 µg / ml gentamycin and were then subdivided into two populations for further three culture days in the presence or absence of 5U / ml rh IL-1β (Genzyme). For cell recovery, alginate beads were finally dissolved into dissolution buffer (55 mM sodiumcitrate, 30 mM EDTA, 0,15 M NACl) and placed at room temperature for 10 min. Viability was checked by eosin-red exclusion and cell number was determined.

Primer syntheses

Arbitrary oligodecamer primers OPA6 to OPA10, OPA16 to OPA20 and degenerate anchored oligo-dT primers ($T_{12}VN$) were synthesized using the 392 DNA synthesizer (Applied Biosystems) and purified by denaturing polyacrylamid gel electrophoresis. Some oligodecamer primers, U1 to U15 were purchased from Biometra (Göttingen, FRG).

List of all degenerate 3' oligo dT-primers [T_{12}VN] used for DDRT-PCR:

Primer	Sequence 5' to 3'
T_{12}VA	5'-TTTTTTTTTTTTVA-3'
T_{12}VA	5'-TTTTTTTTTTTTVT-3'
T_{12}VA	5'-TATTTTTTTTTTVG-3'
T_{12}VA	5'-TTTTTTTTTTTTVC-3'
V = dA, dG, dC; N = dA, dT, dG, dC	

List of all arbitrary 5' oligodecamer primers used for DDRT-PCR:

Primer	Sequence 5' to 3'
OPA 6	GGTCCCTGAC
OPA 7	GAAACGGGTG
OPA 8	GTGACGGGTG
OPA 9	GCGTAACGCC
OPA 10	GTGATCGCAG
OPA 16	AGCCAGCGAA
OPA 17	GACCGCTTGT
OPA 18	AGGTGACCGT
OPA 19	CAAACGTCGG
OPA 20	GTTGCGATCC
U1	TACAACGAGG
U2	TGGATTGGTC
U3	CTTTCTACCC
U4	TTTTGGCTCC
U5	GGAACCAATC
U6	AAACTCCGTC
U7	TCGATACAGG
U8	TGGTAAAGGG
U9	TCGGTCATAG
U10	GGTACTAAGG
U11	TACCTAAGCG
U12	CTGCTTGATG
U13	GTTTTCGCAG
U14	GATCAAGTCC
U15	GATCCAGTAC

RNA isolation and cDNA synthesis

Total RNA from cultured articular chondrocytes was prepared according to a single step method Chomczynski and Sacchi (Chomczynski P & Sacchi N 1987, Anal. Biochem. 162, 156-159) and incubated with 10 U RNasefree DNaseI (Gibco, Eggenstein, FRG) for 30 min at 37°C to remove chromosomal DNA contamination of RNA. After extraction with phenol/choroform (3:1), the supernatant was ethanol precipitated in the presence of 0.3 M NaOAc and RNA was redissolved in DEPC treated water. 0,4 µg total RNA was then reverse transcribed using 200 U M-MLV (Moloney murine leukemia virus) reverse transcriptase (Gibco, Eggenstein, FRG) in a 40 µl reaction volume containing 50 mM Tris-HCl (pH 8,3), 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$, dNTP mix (dATP, dTTP, dCTP, dGTP) of 200 µM each, 40 U RNase inhibitor (Boehringer Mannheim, FRG) and 2,5 mM degnerate oligo-dT primer ($T_{12}VN$) at 37°C for 1 h. Reactions were terminated by heating for 5 min at 95°C.

PCR amplification

cDNAs were amplified in a DNA thermal cycler (Perkin Elmer, model 480) in 20 µl PCR reactions containing 2.5 µM of the original $T_{12}MN$-primer used in cDNA synthesis in combination with 0.5 µM arbitrary upstream primer, dNTP mix (dGTP, dCTP, dTTP) of 0,5 µM each, 10 µCi α-[^{35}S]dATP (1000 Ci/mmole, 10 mCi/ml), 10 mM Tris-HCl (pH 8.3) 50 mM KCl, 1,5 mM MgCl, 0,001 % gelatin and 2.5 U AmpliTaq DNA polymerase. Light mineral oil was overlaid and thermal cycling was performed as follows: 94°C for 30 seconds, 40°C for 2 min and 72°C for 30 seconds for 40 cycles followed by 5 min postextension at 72°C. AmliTaq DNA polymerase was purchased from Perkin-Elmer (Weiterstadt, FRG) and α-[^{35}S]dATP was obtained from Amersham-Buchler (Braunschweig, FRG). After addition of 5 µl stop buffer (95 % formamide, 20 mM EDTA, 0,05 % bromphenolblue and 0,05 % xylene cyanol) radiolabeled PCR-fragments were then displayed on 6 % acrylamide/7 M Urea high resolution sequencing gels of 35 x 43 cm in size; dried gels were exposed to X-ray film (Kodak X-OMAT) and exposed for 48 h, which allows rapid identification of differentially expressed genes by side by side comparison of DDRT-PCR band patterns.

Elution, reamplification and cloning of PCR fragments

PCR fragments identified as differentially expressed bands were cut from acrylamide gels, transferred into Eppendorf tubes and rehydrated for 10 min with 100 µl 10 mM Tris-HCl and 1 mM EDTA at room temperature. After boiling the gel slice for 15 min, the PCR fragment was recovered by ethanol precipitation in the presence of 0.3 M NaAc and 20 µg glycogen as a carrier and redissolved in 10 µl sterile water. 5 µl of this volume was used for reamplification by PCR using appropriate primers and conditions described above except for dNTP concentration of 20 µM and no radioisotope. The reamplified PCR product was visualized by electrophoresis on a 2 % agarose gel and eluted from the gel by ultrafiltration using Ultrafree MC-filters (Millipore). Purified PCR fragments were then cloned into the pCRII-vector (Invitrogen, De Schelp, NL) by the TA cloning method (Kovalic D et al. 1991, Nucleic Acids Research 19, 4640), which allows in-vitro transcription and sequencing from the plasmid.

Sequencing

Plasmid DNA sequencing of subcloned PCR fragments with either SP6(2) or T7(1) primer was carried out using the chain-termination DNA sequencing method, as described by Sanger et al. (Sanger F et al. 1977, Proc. Natl. Acad. Sci. USA 74, 5463-5467.).

Sequence analysis

The sequence analysis revealed the sequences of cDNA clones TAO8/2(2), TAO16/1(2), TAO16/2(2), TAO17(c), TAO19(c), TAU1/1(2), TAU1/1(1), TAU1/2(2), TAU7/1(2), TAU7/1(1), TAU7/2(c), TAU10(1), TAU12/1(2), TAU12/1(1), TAU12/2(1), TAU12/3(2), TAU12/3(1), TAU13/1(1), TAU13/3(2), TAU13/3(1), TCO16/1(c), TCO16/2(c), TCO17(c), TCO18(c), TCU2/1(1), TCU2/2(1), TCU9/1(2), TCU9/2(2), TCU10(2), TCU14(1), TCU14(2), TGO20(2), TGO20(1), TGU5(c), TGU8(2), TGU9/1(2), TGU9/2(2), TGU12(c), TGU13/1(c), TGU13/2(2), TTO16/2(c), TTO20/1(c), TTO20/2(2), TTU2/1(2), TTU2/2(c), TTU3(1), TTU5/1(2), TTU5/2(2), TTU9/1(1), TTU9/2(2), TTU13(2), TTU13(1) disclosed on pages 7 to 14 of the specification.

Searching for homology between subcloned PCR fragments and sequences already listed in one of the DNA databases (GenBank or EMBL database) was performed using the FASTA program developed by Pearson and Lipman (Pearson W & Lipman DJ 1988, Proc. Natl. Acad. Sci. USA 85, 2444-2448) included in the GCG software package (Genetics Computer Group, Madison, USA).

Northern-blot analysis

Cell culture and isolation of RNA was performed exactly as described above. 10 µg of total RNA from both IL-1β stimulated or not stimulated chondrocytes were denatured by heating at 65°C for 10 min in a solution of 50 % formamide, 20 mM MOPS and 2.2 M formaldehyde, separated through a 1 % agarose gel containing 2.2 M formaldehyde in 1 X MOPS and transfered to positively charged nylon membrane (Amersham) by standard blotting procedures [Maniatis et. al 1992]. After UV crosslinking, the blots were prehybridized for 1 h in rapid-hyb-buffer (Amersham) at 65°C. A 330 bp cDNA corresponding to nts 61 to 390 of human osteopontin cDNA (GenBank J04765) and a 340 bp cDNA corresponding to nts 881 to 1220 from human calnexin (GenBank M94859) were radiolabeled for hybridization with α-[^{32}P]dCTP (3000 Ci/mmol, 10 mCi/ ml) using random nonamer primers (Amersham) up to a specific activity of ~ 1,5 x 10^9 dpm / µg DNA. Hybridization was performed for 2,5 h at 65°C in prehybridization solution with 2 ng / ml of labeled probe added. The blot was subsequently washed in 2 X SSC, 0.1 % SDS at 37°C for 15 min (1 X SSC = 0,15 M NaCl, 0.015 M sodium citrate, pH 7,0), followed by two sucessive washes with 1 X SSC , 0.1 % SDS at 65°C for 10 min respectively. If necessary, a final high stringency wash was performed with 0.1 X SSC , 0.1 % SDS at 65°C for 15 min. The blots were then analysed by autoradiography using Kodak X-Omat films at -80°C with intensifying screeens for 2-7 days and intensity of bands was quantified with a phosphorimager (Biorad, model GS-250). All blots were stripped with boiling 0.5 % SDS solution and reprobed with labeled β-actin to demonstrate equal loading of RNA in each lane.

Northern hybridisations (Results)

Fragment TAU7/2(c), identical to TSG-6, was differentially upregulated in IL-1 stimulated cells. This is in concordance with Lee et al. (1992) which reported for TSG-6 a TNF-α and IL-1 mediated upregulation. Fragment TAU1/1, identical to human osteopontin and fragment TTU2/2, identical to human calnexin, both were weaker expressed in IL-1 stimulated chondrocytes compared with the unstimulated cells. To validate our differential display data, we performed Northern analyses of Osteopontin and calnexin expression in IL-1 stimulated and unstimulated chondrocytes originating from a third patient. Both messages were again downregulated. A phosphorimager quantification revealed an osteopontin downregulation by 79% and a calnexin downregulation by 40% in the RNA population from chondrocytes of the third

patient.

Table 1: Current results of differential display reverse transcriptase PCR (DDRT-PCR) to reveal diffential gene expression by chondrocytes with and without IL-1ß

Overview on used primers and number of analysed bands

DDRT-PCR primercombination

3'-Oligo dT-primer (downstreamprimer)	5'-Oligodecamer (upstreamprimer)	putative differential expressed genes by side by side comparison DDRT-PCR band pattern	reproducibility of DDRT-PCR band pattern from first to second, third or fourth DDRT-PCR (same patient[1])	(other patient[2])	eluted from gel and reamplified in PCR	chloned into pCRII vector by TA cloning method verified by PCR	PCR-fragment sequenced using SP6 or T7 promoter
$T_{12}M^{*}A$	OPA 6 - OPA 10	25 out of ~ 4000 bands	7	not done	6	1	1
$T_{12}M^{*}T$	OPA 16 - OPA 20	19 out of ~ 4000 bands	13	9	12	12	12
$T_{12}M^{*}G$	U 1 - U 5	31 out of ~ 4000 bands	not done	11	11	10	10
$T_{12}M^{*}C$	U 6 - U 10	27 out of ~ 4000 bands	not done	13	12	11	11
	U 11 - U 15	21 out of ~ 4000 bands	not done	11	11	10	10
total 4 x = 100 combinations	25	total 123	total	55	total 52	total 44	total 44

* means threefold degeneracy where M may be dA, dG or dC

1 patient female 73 years old diagnosis gonarthosis

2 patient male between 65-75 years old

theoretical consideration:

Suggesting that an arbitrary upstream primer detects 3 % of the total RNAs (Liang 1994), then 97 % of the total mRNAs will not be detected, i.e. with 25 arbitraty oligodecamerprimer and the four degenerate $T_{12}VN$ primers, about half of the mRNAs would be seen ($P = 1-(0,97)^{n} = 1-(0,97)^{25} = 53,3$ %) in 100 lanes of high resolution sequencing gels.

Table 2 IL-1 mediated differentially displayed cDNA fragments of human articular chondrocytes

Fragment	bp	IL-1	Features	Stat.sig.score
TAO 8/2(2)	275 bp	+	146 bp sequenced, no homology found	0.999
TAO 16/1(2)	450 bp	+	80 bp sequenced, no homology found	0.69
TAO 16/2(2)	200 bp	+	115 bp sequenced, no homology found	0.04
TAO 17(c)	412 bp	+	412 bp sequenced, no homology found	0.016
TAO 19(c)	209 bp	--	209 bp sequenced, no homology found	0.99
TAU 1/1(1,2)	450 bp	--	100 % sequence identity to human **osteopontin** cDNA in 303 bp overlap (303 bp seq.)	1.2 x 10-101
TAU 1/2(2)	430 bp	+	188 bp sequenced, no homology found	0.82
TAU 7/1(1,2)	500 bp	+	87 % sequence identity to human cDNA clone **c-1sd02** in 125 bp overlap (235 bp seq.)	8,1 x 10-33
TAU7/2(c)	202 bp	+	99.5 % sequence id to human **TNF stimulated gene-6** in 202 bp overlap	4.8 x 10-76
TAU 10(1)	400 bp	+	181 bp sequenced, no homology found	0,9997
TAU 12/1(1,2)	470 bp	--	319 bp sequenced, no homology found	3.3×10^{-14}
TAU 12/2(1)	390 bp	--	155 bp sequenced, no homology found	0.0078
TAU 12/3(1,2)	250 bp	--	95 % sequence identity to human cDNA clone **HRBBA21** similar to S10 in 158 bp overlap (162 bp seq.)	1.0×10^{-28}
TAU 13/1(1)	600 bp	+	145 bp sequenced , no homology found	0.12
TAU 13/3(1,2)	500 bp	--	439 bp sequenced, no homology found	0.33
TCO 16/1(c)	241 bp	+	241 bp sequenced, no homology found	2.4×10^{-7}
TCO 16/2(c)	230 bp	+	230 bp sequenced, no homology found	4.3×10^{-5}
TCO 17(c)	169 bp	+	169 bp sequenced, no homology found	0.49
TCO 18(c)	168 bp	+	168 bp sequenced, no homology found	1.3×10^{-6}
TCU 2/1(1)	400 bp	+	178 bp sequenced, no homology found	0,66
TCU 2/2(1)	210 bp	+	151 bp sequenced, no homology found	0.0074
TCU 9/1(2)	430 bp	+	99 % sequence identity to human cDNA clone **131036 3'** in 155 bp overlap (155 bp seq.)	$7,2 \times 10^{-58}$
TCU 9/2(2)	320 bp	--	188 bp sequenced, no homology found	0,22
TCU 10(2)	320 bp	--	100 % sequence identity to human cDNA clone **26518 3'** in 85 bp overlap (91 bp seq.)	$2,9 \times 10^{-28}$

Fragment	bp	IL-1	Features	Stat.sig.score
TCU 14(1,2)	280 bp	+	99.3 % sequence identity to human cDNA **HL60 3'directed MboI** in 249 bp overlap (249 bp seq.)	$3,5 \times 10^{-91}$
TGO 20(1,2)	300 bp	+	304 bp sequenced, no homology found	0.95
TGU 5(c)	317 bp	+	317 bp sequenced, no homology found	0.088
TGU 8(2)	320 bp	+	100 % sequence identity to human **28S rRNA** in 58 bp overlap (58 bp seq.)	1.4×10^{-16}
TGU 9/1(2)	280 bp	+	169 bp sequenced, no homology found	0,55
TGU 9/2(2)	220 bp	--	100 % sequence identity to human cDNA clone **12A10B** in 100 bp overlap (173 bp seq.)	4.0×10^{-36}
TGU 12(c)	208 bp	--	87 % sequence identity to human cDNA clone **113442 3'** in 208 bp overlap	5.5×10^{-63}
TGU 13/1(c)	322 bp	+	322 bp sequenced, no homology found	6.9×10^{-13}
TGU 13/2(2)	300 bp	--	94.9 % sequence identity to human **F1 ATPase β-subunit** in 137 bp overlap (137 bp seq.)	2.3×10^{-43}
TTO 16/2(c)	239 bp	+	97.5 % sequence identity to human **ERCC5** in 239 bp overlap (239 bp seq.)	9.3×10^{-88}
TTO 20/1(c)	314 bp	+	100 % sequence identity to human **fibronectin** in 314bp overlap (314 bp seq.)	1.9×10^{-121}
TTO 20/2(2)	400 bp	+	152 bp sequenced, no homology found	0.035
TTU 2/1(2)	300 bp	--	100 % sequence identity to human cDNA clone **118470 5'** in 146 bp overlap (146 bp seq.)	$2,1 \times 10^{-36}$
TTU 2/2(c)	184 bp	--	99 % sequence identity to human **calnexin** in 184 bp overlap (184 bp seq.)	2.3×10^{-84}
TTU3(1)	400 bp	+	97 % sequence identity to human **NADH-DH mtDNA subunit** in 203 bp overlap (203 bp seq.)	8.6×10^{-69}
TTU 5/1(2)	300 bp	--	147 bp sequenced, no homology found	0.0065
TTU 5/2(2)	270 bp	--	118 bp sequenced, no homology found	0,035

Fragment	bp	IL-1	Features	Stat.sig.score
TTU 9/1(1)	350 bp	+	94 % sequence identity to human cDNA clone 83764 3' in 159 bp overlap (159 bp seq.)	$5,9 \times 10^{-23}$
TTU 9/2(2)	320 bp	--	149 bp sequenced, no homology found	0,22
TTU 13(1,2)	350 bp	+	194 bp sequenced, no homology found	0,57

Thus, the 44 identified fragments can be subdivided as follows:

1) 2 fragments with sequence homologies to known human genes with known roles in IL-1 mediated processes:

TAU 7/2 identical with human TNF-stimulated gene-6
TTO 20/1 identical with human fibronectin

2) 6 fragments with sequence homologies to known human genes, whose function in IL-1 mediated processes can be speculated:

TAU 1/1 identical with human osteopontin
TGU 8 identical with human 28S ribosomal RNA gene
TGU 13/2 identical with human F1 ATPase β-subunit
TTO 16/2 identical with human ERCC5
TTU 2/2 identical with human calnexin
TTU 3 identical with human NADH-DH mtDNA subunit

3) 9 fragments with sequence homologies to human genes, identified in human geneome sequencing projects:

TAU 7/1 identical with human cDNA clone c-1sd02
TAU 12/3 identical with human cDNA clone HRBBA21
TCU 9/1 identical with human cDNA clone 131036 3'
TCU 10 identical with human cDNA clone 26518 3'
TCU 14 identical with human cDNA clone HL60 3' directed MboI
TGU 9/2 identical with human cDNA clone 12A10B
TGU 12 identical with human cDNA clone 113442 3'
TTU 2/1 identical with human cDNA clone 118470 5'
TTU 9/1 identical with human cDNA clone 83764 3'

4) 27 fragments without sequence homologies to known human genes The detection of TSG-6 and fibronectin, both genes known to be upregulated by IL-1, points to the importance of those other cDNA fragments in the light of IL-1 mediated processes. Those genes very likely play roles in degenerate joint diseases, including rheumatoid and osteoarthritis and with this are interesting candidates as markers for clinical studies or as drug targets for pharmacological intervention.

Claims

1. Use of osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β a mediated diseases of connective tissues, in particular osteoarthritis.

2. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

3. Pharmaceutical for the prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

4. Use of calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis.

5. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.

6. Pharmaceutical for the prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.

7. Use of TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis.

8. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

9. Pharmaceutical for the prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

10. DNA containing a DNA selected from the group consisting of

TAO8/2(2)

```
       1  CCAAGTTTTT CCAGCAACCC CAAGGGAATA CAGGGAGATC AATGCACCCA
      51  AAATGGGAAA AGAAAAATAC TTCGATGCAA TGAAACAAAG CCTTTTTCCG
     101  TTCAGTTTCC ATAATTCAGT GGTCAGTTTT AAGGCTGCCA CTTGGG
```

TAO16/1(2)

```
       1  GACACGAACA CCACATATTT TTATTGGAGG CCCCATGGCT CCTTGGAAGC
      51  CATTTTGGAA CCAAGGGGAC CCACCTTTTT
```

TAO16/2(2)

```
       1  CTAAATATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC
      51  TAAAAGGATA AGGAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC
     101  ATACTTGCTT TTTAG
```

TAO17(C)

```
       1  CATGAAATAT TTCTTGAGGT AATAAGCTTT TACCAAGCTT ATATTTTTGG
      51  GCAATTCAGT TACAATGAGA AAAAAACACA CCAAAAGACC AAAAATTTTA
     101  AAAACTCACT TTTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT
     151  CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAATA CGATGCAATT
     201  TTAATAAGAA TTTGAAGAAT GACATTAAAT GCTGTCTGAA GCCTTTGTAT
     251  TTTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA
     301  AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTTAAC
```

```
      351  AGATATGATG ACAGNAAAAA TTGATTGCAT ATCTTTTTGC ACTAAAACTT
      401  TTATATTTAT TT

TAO19(C)
        1  AGAGCAGGGG TATTTCNCGG TTCATACCGC CATGGCTTAA GAAGCAAAAG
       51  TCATATACCT TAGTAGTGGC AAAGATNGAG GAGATAAAAA AGAGCCTACC
      101  CAAGCTGTTG TTGAAGAACA GGTCTTAGAT AAAGAGGAAC CCTTCCAGAA
      151  GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA
      201  AGAGATTAA

TAU 1/1(2)
        1  CTAAATGCAA AGTGAGAAAT TGTATTTTTT CTCCTTTTAA TTGACCTCAG
       51  AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTTCAG GTGTTTATCT
      101  TCTTCCTTAC TTTTGGGG

TAU 1/1(1)
        1  ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC
       51  ATCCCCGTTT CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG
      101  GAAGGACAGT TATGAAACGA GTCAGCTGGA TGACCAGAGT GCTGAAACCC
      151  ACAGCCACAA GCAGTCCAGA TTATATAAGC GGAAA

TAU1/2(2)
        1  CCGGAATGGG GAGCAAACTA TAAGAACCGG GACCAGTTTC CTCTCTTTGT
       51  GCCCTAGTTC CCCCTCCTTT GTATACACCC TCCATCCTGA ATAGACTCTG
      101  GTTCTCAGCG TAACACCGAC AACATTCAAT CCTGTAGAGA AACAAATGTT
      151  AGCTCAGAAG GACACAGCCT TTGAATCATC AGAGAGTT

TAU 7/1(2)
        1  GTTAAGAATA ACTAAATAAA AGTTTTAATT AATTTAGGAA TATAAAAAAC
       51  TATTAACATT TAATTTTATA ACTGTATCTG CCAAGCAACT TTAAATATAA
      101  TTTATTTACC

TAU 7/1(1)
        1  CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGGAAAC CATCCCCTCA
       51  AGCATTTATC CTTTGAGTTA CAAGCAATCC AATTACACTC TTTTAGTTAT
      101  TTTTAAATGT ACAGTTAGGT TATTA

TAU 7/2(C)
        1  CCTTGAAGAT GACCCAGGTT NCTTGGCTGA TTATGTTGAA ATATATGACA
       51  GTTACGATGA TGTCCATGGC TTTGTGGGAA GATACTGTGG AGATGAGCTT
      101  CCAGATGACA TCATCAGTAC AGGAAATGTC ATGACCTTGA AGTTTCTAAG
      151  TGATGCTTCA GTGACAGCTG GAGGTTTCCA AATCAAATAT GTTGCAATGG
      201  AT
```

TAU10(1)

```
   1  GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA
  51  CTATAATTTT ACAAAGTTAA ATTTATAAGC TAGCATTAAG TAAAGTGAAG
 101  TTCCAGCTCC CTTGCTAAAA ATAACTAGAG GTAATAATTG GTATTCAGGT
 151  AACTCATTTA CATCATAATG TGTTGTGAAA A
```

TAU12/1(2)

```
   1  TATAAAATAT AAATTATATT ATAAATCATG TATTATTTAT AAAATTATAT
  51  TATAAATTTA TAAAAATATA AATTATATTT TAGGCTTAAT GTATAAGGAA
 101  TATAAATTAT TAATAAGCAT ATGA
```

TAU 12/1(1)

```
   1  TGTAATTAAC TGTNCTTGTA GGTCTGTCTT TTATACATGT GTGAGTTTTT
  51  CTTTACAATA GATTCCTAGC ATTGGGATTG CTAGGTCAGA TGGTATGCAC
 101  ATTTGACATT TTGATTGATA GCACCAGATT GCTTTGTTAA AAAATTTTNN
 151  TTTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC
```

TAU 12/2(1)

```
   1  GGGAAGTGAA TTGAAAATAC TTCTTTNTCA ACATAATTTT NGGGTTTTGA
  51  AATTGTGTTT GGGTTTTCAG GAAATTGGTG GTAATCTTGT ATTAGCTGAA
 101  AAAAAGTGAA TTTTAAAATT CTCAGTGAAG AAGCAAATGA TTTATTTTTC
 151  ATAGA
```

TAU12/3(2)

```
   1  TGTTCTGGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCT
  51  TTCAGGTAAG
```

TAU12/3(1)

```
   1  CTAAAGAACT TGGTATCTCT ATTAAAGCAC ACGAACCTCC AAGGAAAATA
  51  GAGCGATTTA CTCTTCTCAT ATCAGTGCAT ATTTATAAGA AGCACGGAGT
 101  CA
```

TAU13/1(1)

```
   1  AGTCATCAAT TCCTTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAAA
  51  GTAATTATAA GGTGTTATAT TGCATATAAT CAGAAAACTA AATGGAAATA
 101  AAATTTTAGT AAGCCCGGCC CCTTTGACCG ATACAGAAAA CTTGA
```

TAU 13/3(2)

```
   1  TATATGGCAG TCTAAAGCAT CAAAGATTTG CATCAACATC TTTCATTTTA
  51  GACATCTCCT TGCAATGTAA AATATCATGT ATCAACAACA TCTGGTGCAA
 101  ATCCATGAGT CTAACTCGAC ATTCATCTTA GCTCGATTAT TATTCCTTCG
 151  TACAGTCGAT GTAAACAATA CAGAAAGAGG ATTATTAAGA ACAGTTT
```

TAU 13/3(1)

```
   1  ATTCATGAAA TGGTCTATAT GCATGATATT GTAAATTCGG ACTCGAAACC
  51  GAAACCAAGG ATTCCGTTAC AAAAATTCCT TAATGCTGAG AATGTTCTCA
 101  CGCAAACAAC ATCATGGACA TTAAATTCAA GATATGTGAA TGTTAATTCT
 151  GTCAATAAAG TCAACGTAAA GAGTAAAGTT AAAAACAGTT ATATCTNNNC
 201  TGTCAATGAT GAGTTTAGTT TAACAGATGA TGAATCAATT CT
```

TCO 16/1(C)

```
   1  CAAAGTGTTT TTGGTTTTGA GAGAGAGAGA GATTGAGAGA CAGAGAGAGA
  51  GAGAGAAACC AAGGGATCAT GATAGTTATA GTCAAATACG AGGTTGGATT
 101  ATCTTTTGAA AATGTGTTGG TTCTGTGATA CAAGAGGAAG CTAAGACATA
 151  TCGTGGAAAC ATCTCCCCCC TCCACCTTAA TATCAAGAAC AAATTGTGGA
 201  ATCTAATGTT AATGAGAAGT AGTTCCCCAC TGTGTCAGAT G
```

TCO16/2(C)

```
   1  NCATCTGACA CAGTGGGGAA CTACTTCTCA TTAACATTAG ATTCCACAAT
  51  TTNNNCTTGA TATTAAGGNN NNNNNGGGAG ATCGTTTCAC GATATCGTCT
 101  TAGCTTCCTC TTGTATCACA GAACCAACAC ATTTCAAAAG ATAATCCTTC
 151  CTCNNTTTGA CTATAACTAT CATGATCCCT TGGTTCTCTC TCTCTCTCTG
 201  CTCTCTCATC TCTCTCTCTC TNAAAACNAA
```

TCO17(C)

```
   1  ACAGTAGTTA GGAGTTTCTT TACTTACAAA ATCACTGGAA ATGATTAAAT
  51  TGCTTTTCCC CCTCCCCAGA GGTGCATTTT TCTTATTTCC ATATAGTAAA
 101  GTTGAGCTTT TACAGTGCAT AATGTGACAT TTGGAATGCT TATCAACTGC
 151  ATGTAAACAT TAATAACCT
```

TCO18(C)

```
   1  GTAAATGGTA TTANNNGCTG AAGAAAAAAA ATTTTTCAAG ACCTCTGTTT
  51  TTTAACTGAA CTTTATCATT GGCATTGTGG GCTTTGAAGT TGCTGGGATA
 101  AATTAATATA ATTAAATAAA AGACTGAATT TAATTGCAAA AAAAAAAAAA
 151  AACAATAAGT GTGGTGAT
```

TCU2/1(1)

```
   1  AAGAAATTAT CCAGTTATTT ACAAGGCCAC TGATATTTTA AACGTCCAAA
  51  AGTTTGTTTA AATGGGCTGT TACCGCTGAG AATGATGAGG ATGAGAATGA
 101  TGGTTGAAGG TTACATTTTA GGAAATGAAG AAACTTAGAA AATTAATATA
 151  AAGACAGTGA TAAATACAAA GAAGATTT
```

TCU2/2(1)

```
   1  CGGGTTAATA TTATCCTCTA GTATAAGTGA ATTACTAGTT TCTCTTTATT
  51  TAGACAAACA CACACACACC AGATAATATA AACTTAATAA ATTATCTGTT
 101  AATGTAGATT TTATTTAAAA AACTATATTT GAACATTGGT CTTTCTTGGA
 151  C
```

TCU9/1(2)

```
       1  ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTTG TTTACAAAGA
      51  AAGTCTAAAA TTTCAAGAAC ATTCAAAGAG CTAACACAGT AAAGGTCATG
     101  CAAGTTCTAG AATAGTGAAT CATGACAGAA CTCATTCATT TTATCCTTTA
     151  TCTCC
```

TCU9/2(2)

```
       1  AAGTATGGGT AGCTAAATTT GCATTAAATT AAAAGTACAT ATAATGCAAC
      51  ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTGTACTA CACACCCTTA
     101  AAATGTTTTT CAAAGTCTTA ATATATTAGA ACATGTTTTC ATTTTTTCAT
     151  GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG
```

TCU10(2)

```
       1  AATACAGTTA TTCTAGCTTT TCATATTCAA TTTGAATGAT CAGAAAAGTA
      51  TATTAGTCAC ACAGAATTAA ATATTTTAGA TAGTAAGAAT C
```

TCU14(1)

```
       1  ATCCTTAGTA AGTGGATTTT GGGGAAAAAA GCACCTGGGC TTCTGGTTCT
      51  TTTTGATAAT ATATAAAATT ATTCATTATG AGGTTGCAGT TGTTTGCAAA
```

TCU14(2)

```
       1  GAAGTGAAAG TCAGCCCTTT AGCTATTATT TATTGCTTTA TTAGAGCAGA
      51  GGGAAGTGAC ACTCATTGCC TTCACAGAGC TCTGCAGAAA TATATGCACA
     101  GAGTGGTCAA TGCCAACATC TGAGTAAGTC TTCCAAA
```

TGO20(2)

```
       1  CAGAACATTA GGATTTATTC CTTGATTAGT TCAAATGATT TCAACAGCTG
      51  AATTCCTTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC
     101  TGAAACTTCC TATAACTGTA GTGATATGTA CACAGCTACA TAGCAAAGTG
     151  CTTCATTATG AAAATGAAGA A
```

TGO20(1)

```
       1  CAGTGTGAGA GTCTCATTTC TATGCACAGT GTTTCTCAGG AGGATGGAGC
      51  TAGTTAGCTG TCTGTTGTCT GTAGCCCAGC TTGATAATGG AACTATACAG
     101  CGAAGAGACA ATCTCTGGCA AGTTTTTGTA GAA
```

TGU5(C)

```
       1  TTAGAGTAAA ATTCCAAATA AATGCTTTGC TCCAAAATTA CACTAACCAG
      51  GCTGGGTCTC TATCATACAT CTTCAATACC CTCAAACCTA GATTGTAAAG
     101  TGAAAAAAGT GATTAGCNNT TCCATTTGTT CATTCTGTCA CTCACATTCT
     151  TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTA
     201  GCCCCCTGGA GGTTACATAG TTATAATTTA GTCTTCAGAA TCCGTTCGAA
     251  GGGNNNNGTT ACTATTTTTA AGATAATTAG AACCCACCTT GTAGCAATAA
     301  AAGTTTTCTT GTCTTTG
```

TGU8(2)

```
       1  GCGAAAGACT AATCGAACCA TCTAGTAGCT GGTTCCCTCC GAAGTTTCCC
      51  TCAGGATA
```

TGU9/1(2)

```
       1  TTAATGTTTA AATACTACTT TTTTTTCAAG CTTGCCCTAG ATACCAACTG
      51  TTTATCTAAC ACACAATTCC AGTGTTGCCA AGCCTCATGC CAATTTGAAG
     101  GGAACAGCCA AAACTTATGC ATTCATATAA AAAGAGTCTC TAGGCTCTTA
     151  TATCTACATT ATAATTTTT
```

TGU9/2(2)

```
       1  GGAATAACAT TTTTTTATGA GGGAACCCTT TAAAATGGAT GCACACAGTG
      51  GCATTTTCTC CTAGGCTCAA AGCTGAGTAC ACTCCCGTAA TTTTAATAAT
     101  ATTTTAGGCA AGTCCTATGA CAATTATACC AACAAGTTTC TTCAACCCCA
     151  CCACCACCCC ACCATCTCTA TGC
```

TGU12(C)

```
       1  GGAGGAAGCT TTATTTGGGA AGAGTGCGGT TCNNTCGGCC CTGATCAGCT
      51  CTAGCCTGCC CACCCCATCT CAGCCAGGCG GCTTTACTTC TTCCTGAGCT
     101  TCAGGTCTTT CTTCTTCCTG ATTTCCTTGG CCAGCTCCCC AATCAATCTC
     151  CAGTACTCAT TGAACTTGAG CTCCGAGNCC TGATTCACAT CCAAGCTCTT
     201  CATCTTCT
```

TGU13/1(C)

```
       1  GGATGTGGTA GTTGATCTTT AATGCCCATT CTAGGTCGGA AAAATCCATG
      51  ATCCTAACTT TTAAGAGAAG GTTGGTAACT CTACTTAGGA CTTTTTTTTG
     101  TAAGAGGAAT AATGTAGCCT CACCCTTATC TTTCTGGAAA TGTTTAAACC
     151  ACTGAAATAT GGAGATCAAA TCCAGCTTAC ACACTGGTAA CTCAAATACT
     201  ATTTTTTTTT TAAACTATCT TTTCTAAACT AATCACCCCT CTTGTACATA
     251  GAACTTTCTA TCTCAGTGCC AATTCTTAGA GGTTGATGCA AACAGCTCTC
     301  CAGAGAGCCT GTGCTATTGT TC
```

TGU13/2(2)

```
       1  GGGGTGTACA TTTTATTGGA AACCTTAAAT ACTGTTCAGA AAGAATATAT
      51  CTTCAATCAA GGTCTTGCCG AGCCTACACA GAAAAATGAA GCTTTTTGGG
     101  TTAGGGGCAA GGATATATAC AGTACAGAGG ACAAAGA
```

TTO16/2(C)

```
       1  ACATTCATTA AAGATGAACT TTCAGCATCT TCACTTGAAG ATCCATCAGA
      51  TGATTCTGAG AGGCAGGTCT CCCCCAAAAA TCCACCGCAT GTATTCTTTC
     101  GTTTAGAATC TGAAAGCCTC TTTCTTTTCA GGCTTGATGA CTCTTCTAAG
     151  GTATTTGTTA TGCCTCTCTT CTGGGTTTTT CGTTTTGCCT TATCAAGTAG
     201  CTNAAATTCA AACACCATGG CAANAGAAAC TGCTTCTAT
```

TTO20/1(C)

```
       1  CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT
      51  TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT
     101  CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAAACCTG
     151  GAGTTGATTA TACCATCACT GTGTATGCTG TCACTGGCCG TGGAGACAGC
     201  CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA
     251  ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACTGT TTTAATAAAA
     301  GATTTACATT CCAC
```

TTO20/2(2)

```
       1  TTGGTACCAC AGTCACAGAA CTGGGGGTCA TTTTCTAGAT GAAACAAACG
      51  GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCCTC
     101  CATGAATTTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA
     151  AG
```

TTU2/1(2)

```
       1  CTAGAACTTC CAAAGGCTGC TTGTCATAGA AGCCATTGCA TCTATAAAGC
      51  AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC
     101  ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG
```

TTU 2/2(C)

```
       1  AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT
      51  TGTTTTCTTA TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG
     101  CATGTTTTTC TTCATAGATA CCCGTTTTGG GGTTTTTGCG TCGGAAGATG
     151  AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG
```

TTU3(1)

```
       1  GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCGG
      51  GAGGACCTAT TGGTGCGGGG GCTTTGTATG ATTATGGGCG TTGATTAGTA
     101  GTAGTTACTG GTTGAACATT GTTTGTTGGT GTATATATTG TAATTGAGAT
     151  TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG
     201  AAG
```

TTU 5/1(2)

```
       1  GACAAAAAAA AAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA
      51  GTATCTTAAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA
     101  GCTGAAAACT TAAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG
```

TTU5/2(2)

```
       1  GCATCCATTG TACATTGTTT GGTTTGAGGT TACCATGAGG CCTGTAAATA
      51  CTATCTTATA ATTTATTATT TCAACCTGAT AAAACTTAAC ACTATTTGCA
     101  TAAACAAACA AACGAAAA
```

```
TTU9/1(1)
       1  TAAAATACTG GTTCTTTTAT TCTGCAATAT TTTTAAAAAT CACATTTTCA
      51  GCCAGGCGCA GTTTCCCACA CCTGTAATCC GGCACTTTGG GAGGCTGAGA
     101  TGGGTGGATC ACAAGGTAGG AGATCAAACA TCCTGGCCAA CATGGTGAAC
     151  CTGTTTACT

TTU9/2(2)
       1  CAAGTATGGG TAGCTAAATT TGCATTAAAT TAAAAGTACA TATAATGCAA
      51  CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCTT
     101  AAATGTTTCA AAGCTTAATA TATTAGAACA TGTTTTCATT TTCAGGGAG

TTU13(2)
       1  GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG
      51  TAAAATGAAA GAAATCATTT TTAACTCCTA AGATGT

TTU13(1)
       1  TGAATTAAAT GGACTCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT
      51  AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT
     101  CTTCTTTT
```

or an analog thereof.

11. Vector containing a DNA according to claim 10.

12. Host cell containing a vector according to claim 11.

13. Method for isolating a gene inducable by treating chondrocytes with IL-1β containing the steps:

(a) hybridizing a DNA according to claim 10 under stringent conditions against DNA or RNA containing said gene; and
(b) isolating said gene.

14. A method according to claim 13 wherein said DNA or RNA has been isolated from chondrocytes, particularly human chondrocytes, that were treated with IL-1β.

15. Process for expressing a gene isolated according to claims 13 or 14 containing the steps:

(a) cloning said gene into a suitable expression vector; and
(b) expressing said gene in a suitable host cell.

16. Method for producing a protein containing the steps:

(a) culturing a suitable host cell containing a vector which contains a DNA according to claim 10 or a gene produced by a method according to claim 13 or 14; and
(b) isolating the expressed protein.

17. Diagnostic aid containing a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof.

18. Use of a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof for the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

19. Use of a gene isolated according to claim 13 to 14 for the production of a pharmaceutical.